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# Bovine cytotoxic T-cell clones specific for cells infected with the protozoan parasite *Theileria parva*: Parasite strain specificity and class I major histocompatibility complex restriction

(East Coast fever/cell-mediated immunity/immunoprecipitation/cattle)

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**ABSTRACT** We present information on the specificity of three bovine cytotoxic T-cell clones reactive with lymphoblasts infected with the protozoan parasite *Theileria parva*. The clones were derived from peripheral blood mononuclear cells of an animal immunized with *T. parva* (Muguga stock), after five stimulations *in vitro* with an autologous parasitized cell line. The three clones belonged to the BoT8<sup>+</sup> subset of T cells, which is similar to the human CD8<sup>+</sup> T-cell subset. On the basis of analysis on a panel of infected target cells originating from cattle of different major histocompatibility complex (MHC) phenotypes, killing by all three clones was found to be restricted to targets bearing the class I MHC specificity KN104, which is defined by alloantiserum KNA104 and monoclonal antibody IL-A4. This class I MHC restriction was confirmed by blocking of target cell lysis with these antibodies and monoclonal antibody w6/32, which reacts with a nonpolymorphic determinant on bovine class I MHC molecules. The three clones were parasite strain specific, in that they did not kill cells of the appropriate MHC type infected with *T. parva* (Marikébuni stock). These findings, taken together with previous observations that immunization of cattle with *T. parva* (Muguga) does not provide protection against challenge with *T. parva* (Marikébuni), suggest that the cytotoxic T cells recognize a cell surface antigen that may be important in induction of immunity to the parasite.

The tick-borne protozoan parasite *Theileria parva* causes an acute, usually fatal, disease of cattle, known as East Coast fever, which results in major economic losses throughout East and Central Africa. The parasite enters bovine lymphocytes, which subsequently undergo blast transformation and multiplication. Parasite and host cell divide synchronously (1), resulting in rapid clonal expansion of the parasitized cells and high levels of parasitosis in the lymphoid tissues of infected cattle.

Cattle that recover spontaneously from infection or are immunized by infection and treatment regimes are immune to challenge with the homologous stock of the parasite but may be susceptible to challenge with heterologous stocks (2–5). The immunological basis of this parasite strain heterogeneity is poorly understood. Evidence from studies of cattle undergoing immunization suggests that cell-mediated immune responses to parasitized lymphoblasts are important in immunity (reviewed in ref. 6). Genetically restricted cytotoxic cells specific for parasitized targets are detected transiently in the peripheral blood of immune cattle (6–8). Preliminary observations have indicated that these cytotoxic cell populations have a degree of parasite strain specificity (9).

Further analysis of the specificity of the cytotoxic response and its role in immunity has been hampered by difficulties in

generating effector cells *in vitro* with specificity similar to that of those arising *in vivo*. Cytotoxic cells have been induced in autologous *Theileria* mixed leukocyte cultures (MLCs), in which peripheral blood mononuclear cells (PBM) are stimulated by autologous infected cells, but invariably they contained a major component that was not specific for parasitized targets (10–12). By repeated stimulation in autologous *Theileria* MLCs, we have obtained populations of T cells that are highly enriched for *Theileria*-specific cytotoxic cells. We report here findings on the MHC restriction and parasite strain specificity of three *Theileria*-specific cytotoxic T-cell clones derived from one such culture.

## MATERIALS AND METHODS

**Cattle.** Donors of PBM were Boran (*Bos indicus*), Friesian, Ayrshire, or Hereford (*Bos taurus*) female or castrated male cattle, ranging from 6 to 48 months of age. The animal from which cytotoxic T cells were derived was a 3-year-old Boran steer (B641). All animals were clinically normal and were serologically negative for antibodies to *Theileria parva* schizonts at the outset of the experiments (13).

**Typing for Major Histocompatibility Complex (MHC) Antigens.** Class I MHC allelic products of the bovine lymphocyte antigen (BoLA) A locus (14) were defined in a microlymphocytotoxicity test using a panel of alloantisera (15). Animal B641 had the BoLA-A phenotype w10/KN18. In all cases, PBM and their derivative cell lines had the same MHC phenotype.

**Cell Lines.** Lymphoblastoid cell lines infected with *T. parva* were established from PBM or cloned T cells by infection *in vitro* with sporozoites from tick salivary glands (16). The sporozoites used were from the Muguga and Marikébuni stocks of *T. parva*, which are known to differ on the basis of cross-protection (5). Some of the infected cell lines were cloned in limiting-dilution cultures. Culture medium consisted of RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (GIBCO, Uxbridge, U.K.), 2 mM L-glutamine, gentamycin at 50 µg/ml, and 50 µM 2-mercaptoethanol. Confirmation that cell lines were infected with particular parasite stocks was made by typing the intracellular schizont with parasite strain-specific monoclonal antibodies (mAbs) (17).

To obtain uninfected lymphoblasts for use as target cells in cytotoxicity assays, PBM were stimulated once by concanavalin A (Con A) and then maintained by regular addition of 20% T-cell growth factor (TCGF). The TCGF consisted of conditioned medium from bovine PBM stimulated by Con A

Abbreviations: MHC, major histocompatibility complex; MLC, mixed leukocyte culture; BoLA, bovine lymphocyte antigen; PBM, peripheral blood mononuclear cells; mAb, monoclonal antibody; TCGF, T-cell growth factor.

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(18). One day before use, the cells were given 20% TCGF containing 0.1 M methyl  $\alpha$ -D-mannoside (Sigma; grade III).

**Generation of Cytotoxic Cells in an Autologous *Theileria* MLC.** Animal B641 was immunized against *T. parva* (Muguga) by inoculation of sporozoites and treatment with long-acting oxytetracycline (4). Ninety-five weeks after immunization, an autologous *Theileria* MLC was established from the animal. PBM were obtained from venous blood collected into Alsever's solution as described previously (18). Aliquots of  $4 \times 10^6$  PBM and  $2 \times 10^5$   $\gamma$ -irradiated [5000 rads (1 rad = 0.01 gray) from a  $^{137}\text{Cs}$  source] autologous *T. parva* (Muguga)-infected stimulator cells were added to the wells of 24-well cluster plates (Costar, Cambridge, MA) in a total volume of 2 ml of culture medium. After 7 days, cells were harvested from the wells, and viable cells were separated on Ficoll-Paque (Pharmacia) and restimulated with irradiated autologous parasitized cells as in the primary culture. The cells were similarly stimulated with irradiated parasitized autologous cells for a third, fourth, and fifth time at weekly intervals, except that at the third stimulation,  $2 \times 10^6$  responder cells and  $2 \times 10^6$   $\gamma$ -irradiated autologous PBM as filler cells were added per well and at the fourth and fifth stimulations  $1 \times 10^6$  responder cells and  $3 \times 10^6$  filler cells were added per well.

**Cloning and Screening of Cytotoxic Cells.** Clones were derived from the autologous *Theileria* MLC 7 days after the fifth *in vitro* stimulation. To increase the frequency of clones restricted by class I MHC molecules, the parent cell population was depleted of BoT4<sup>+</sup> lymphocytes by complement-mediated lysis with mAb IL-A12 as described previously (19). The cells were resuspended at  $10^6$  per ml in culture medium supplemented with 10% TCGF and maintained at 39°C overnight prior to cloning. Cells were cloned by limiting dilution in 96-well round-bottom culture plates (Costar): cells were distributed at 0.3 and 1 cell per well with  $1 \times 10^3$   $\gamma$ -irradiated autologous infected cells as stimulators and  $2 \times 10^4$   $\gamma$ -irradiated autologous PBM as fillers, in a final volume of 200  $\mu$ l of culture medium supplemented with 20% TCGF containing 0.1 M methyl  $\alpha$ -D-mannoside. After 2 weeks, wells with cell growth were identified. Rapidly growing clones were split into six round-bottom wells and restimulated as for initial cloning, while slow-growing clones were restimulated in the original well to be split and propagated later.

One week after splitting, clones were tested for cytotoxic activity and positive clones were expanded further in 96-well round-bottom plates. After 1 week, cells were transferred to 24-well cluster plates:  $2 \times 10^5$  effector cells and  $2 \times 10^6$  irradiated stimulator cells were added per well in 2 ml of culture medium containing 20% TCGF. The cytotoxic cells were subsequently maintained in 24-well cluster plates by similar stimulations every 2 weeks. Cell numbers increased 5- to 50-fold over a period of 2 weeks.

**Cytotoxicity Assays.** An indium-111 ( $^{111}\text{In}$ ) release assay (20) was used for initial screening of clones for cytotoxicity. Target cells, obtained from cultures in exponential growth phase, were resuspended in cytotoxicity medium and labeled with [ $^{111}\text{In}$ ]indium oxine (Amersham International, Amersham, U.K.; code IN.15P). Aliquots (50  $\mu$ l) containing  $10^6$  cells were incubated with 5  $\mu\text{Ci}$  (1 Ci = 37 GBq) of [ $^{111}\text{In}$ ]indium oxine at 39°C for 15 min. The labeled cells were washed six times in 6 ml of cytotoxicity medium. Effectors from 1 well of a 96-well round-bottom plate (estimated to contain  $5$ – $10 \times 10^3$  cells) were screened on two targets, namely autologous uninfected lymphoblasts and autologous cells infected with *T. parva* (Muguga). The effectors were mixed with  $5 \times 10^3$   $^{111}\text{In}$ -labeled target cells in V-bottom wells of 96-well plates (Flow Laboratories), pelleted by centrifugation (7 min at  $160 \times g$ ), and incubated for 4 hr at 39°C in a humidified atmosphere of 5%  $\text{CO}_2$  in air. The cytotoxicity

medium consisted of RPMI-1640 medium with 10% fetal bovine serum.

The cytotoxic activity of established cloned cells was measured in a 4-hr  $^{51}\text{Cr}$  release assay using  $5 \times 10^4$  target cells per well (18). Percent cytotoxic release of  $^{51}\text{Cr}$  or  $^{111}\text{In}$ , herein referred to as percent cytotoxicity, was calculated as  $100 \times (\text{test release} - \text{spontaneous release}) / (\text{freeze-thaw release} - \text{spontaneous release})$ .

**Blocking Assay.** Several mAbs and bovine alloantisera were tested for their capacity to block cytotoxicity by interaction with either the target cells or the effector cells. For blocking, 100  $\mu$ l of antibody was added either to 50  $\mu$ l of  $^{51}\text{Cr}$ -labeled target cells or to 100  $\mu$ l of effector cells in the wells of 96-well flat-bottom culture plates (Costar) and incubated for 1 hr at 23°C. Aliquots, 100  $\mu$ l of effector cells or 50  $\mu$ l of target cells, were then added, respectively, to the preincubated targets or effectors and plates were incubated for 4 hr at 39°C.

mAbs and alloantisera were used at a final dilution that gave 10 to 100 times saturating levels of binding as assessed by indirect immunofluorescence analysis.

**Alloantisera and mAbs.** Alloantiserum KNA104 defines the KN104 specificity, which is expressed in some cattle with the BoLA-A w10 specificity and some without the w10 specificity. Alloantiserum KNA109 recognizes another BoLA-A specificity not present on KN104<sup>+</sup> animals. mAb IL-A4 (IgM) recognizes a polymorphic MHC determinant restricted to animals expressing the KN104 specificity. mAb w6/32 (IgG2a) defines a monomorphic determinant on human HLA-A, -B, and -C molecules (21) and crossreacts in a nonpolymorphic fashion with bovine class I MHC molecules (22). mAb B1.1G6 (IgG2a) reacts with human and bovine  $\beta_2$ -microglobulin (23, 24). mAb BT3/8 (IgG2a) recognizes a cell surface molecule that is expressed on all cell lines infected with *Theileria* and on some normal bovine leukocytes (25).

mAbs IL-A12 (IgG2a) and IL-A17 (IgG1) identify two distinct subpopulations of T lymphocytes in cattle; these have been termed BoT4<sup>+</sup> (38) and BoT8<sup>+</sup> (39), respectively, because of their similarities to human T-cell populations defined by CD4 and CD8.

**Immunoprecipitation.** Con A-stimulated lymphoblasts were surface-labeled with  $\text{Na}^{125}\text{I}$  by the lactoperoxidase technique (26). Solubilization of labeled cells, immunoprecipitation with *Staphylococcus* protein A-Sepharose, and sodium dodecyl sulfate/polyacrylamide gel electrophoresis of the precipitated samples were carried out as described previously (25), with minor modifications. Immunoprecipitation was performed at pH 7.5, except in the case of mAb IL-A4, which was used at pH 8.5. Molecules reacting with a particular antibody were removed from the lysate prior to precipitation with a second antibody by several rounds of precipitation until the radioactivity in the pellet was equal to that in negative controls.

## RESULTS

**Propagation and Cloning of *Theileria*-Specific Cytotoxic Cells.** No cytotoxic activity was detected in PBM from B641 at the time of initiation of the autologous *Theileria* MLC. However, after five stimulations *in vitro* with autologous irradiated parasitized cells, 95% cytotoxicity was observed at an effector-to-target ratio of 15:1. Killing was restricted to the autologous infected target and those allogenic infected targets that were matched for one or other BoLA-A product. There was no killing of uninfected targets. On cloning of this population, after depletion of BoT4<sup>+</sup> cells, cell growth was detected in 36 out of 768 wells at 0.3 cell per well, and 46 out of 380 wells at 1 cell per well. Cells in 27 wells exhibited cytotoxic activity. Three clones, T15.8, T15.114, and T15.132, restricted to autologous infected cells, were expand-

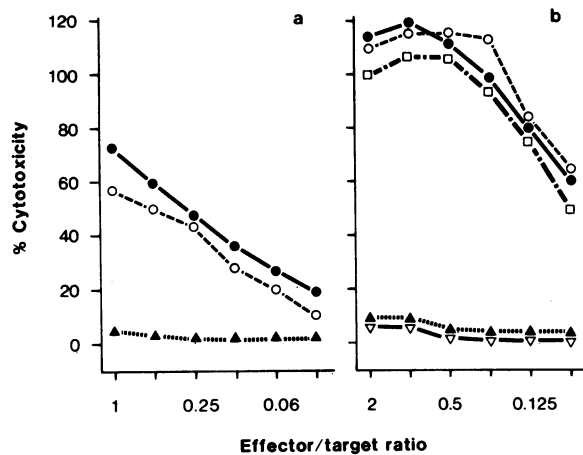


FIG. 1. Blocking of target cell lysis in the cytotoxicity assay by preincubation of target cells with antibodies. Cytotoxic T-cell clone T15.114 was used as an effector against a target cell line infected with *T. parva* (Muguga) from animal C603. Effectors were harvested 2 weeks (a) or 1 week (b) after last stimulation. The following antibodies were used in a: ●, none; ▲, KNA104; ○, KNA109. In b: ●, none; ▲, IL-A4; ○, BT3/8; ▽, w6/32; □, B1.1G6.

ed for further characterization. These clones exhibited maximal killing potency around 7 days after stimulation, giving 100% cytotoxicity at an effector-to-target ratio of 0.25:1 to 0.5:1 (Fig. 1b). When cells were harvested more than 1 week

Table 1. MHC restriction of three *Theileria*-specific cytotoxic clones from animal B641, tested on a panel of bovine cells infected with *T. parva* (Muguga)

| <i>T. parva</i><br>(Muguga)-<br>infected<br>cell line | Target                |                              | Class I MHC phenotype detected |                              |  | % cytotoxicity by clone* |         |         |
|---|-----------------------|------------------------------|--------------------------------|------------------------------|--|--------------------------|---------|---------|
|   | By allo-<br>antiserum | By mAb<br>IL-A4 or<br>KNA104 | By allo-<br>antiserum          | By mAb<br>IL-A4 or<br>KNA104 |  | T15.8                    | T15.114 | T15.132 |
|   |                       |                              |                                |                              |  |                          |         |         |
| B641  | w10/KN18              | +                            |                                |                              |  | 87                       | 81      | 84      |
| C167  | w10/w4                | +                            |                                |                              |  | 102                      | 96      | 99      |
| C234  | w10/w6                | +                            |                                |                              |  | 87                       | 87      | 79      |
| C447  | w10/w7                | +                            |                                |                              |  | 111                      | 113     | 122     |
| ND2   | w10/w20               | +                            |                                |                              |  | 101                      | 101     | 99      |
| B166  | w10/w4                | +                            |                                |                              |  | 117                      | 115     | 111     |
| C603  | w10/-                 | +                            |                                |                              |  | 94                       | 95      | 96      |
| B732  | w10/w6                | -                            |                                |                              |  | 2                        | 3       | 1       |
| C315  | w10/w6                | -                            |                                |                              |  | 1                        | 2       | 1       |
| C538  | w10/KN22              | -                            |                                |                              |  | 0                        | 0       | 0       |
| S823  | w10/w13               | -                            |                                |                              |  | 1                        | 0       | 0       |
| D96   | w10/w6                | -                            |                                |                              |  | 0                        | 0       | 0       |
| C17   | w10/w7                | -                            |                                |                              |  | 0                        | 0       | 0       |
| K240  | w7/-                  | +                            |                                |                              |  | 97                       | 94      | 101     |
| K96   | w8/-                  | +                            |                                |                              |  | 63                       | 62      | 62      |
| K104  | w1/-                  | +                            |                                |                              |  | 116                      | 109     | 108     |
| B171  | KN18/w7               | -                            |                                |                              |  | 3                        | 3       | 2       |
| C481  | KN18/-                | -                            |                                |                              |  | 3                        | 2       | 3       |
| C631  | KN18/-                | -                            |                                |                              |  | 2                        | 2       | 1       |
| C428  | KN18/-                | -                            |                                |                              |  | 2                        | 1       | 2       |
| B353  | w20/-                 | -                            |                                |                              |  | 2                        | 2       | 2       |
| C165  | w6/w7                 | -                            |                                |                              |  | 2                        | 0       | 0       |
| C401  | w6/w4                 | -                            |                                |                              |  | 2                        | 1       | 3       |
| S829  | w13/w16               | -                            |                                |                              |  | 0                        | 0       | 1       |

\*Results are presented for an effector-to-target ratio of 1:1.

after stimulation, higher effector-to-target ratios were required to obtain maximum cytotoxicity (Fig. 1a). The clones have been maintained in culture for 8 months without loss of cytotoxic capacity or specificity.

**MHC Restriction of *Theileria*-Specific Cytotoxic Clones.** Cytotoxic activity of the three clones was tested on several occasions on a total of 32 *T. parva* (Muguga)-infected cell lines from 24 animals. The three clones had similar MHC restriction patterns. Only autologous targets and allogenic targets from animals expressing the KN104 specificity were killed (Table 1). The latter group consisted of some animals expressing the w10 specificity and some animals expressing neither the w10 nor the KN18 specificity.

**Parasite Restriction and Strain Specificity of the Cytotoxic Clones.** The three cytotoxic clones did not kill uninfected autologous and allogenic targets from nine different animals expressing the KN104 specificity. They killed target cells infected with *T. parva* (Muguga) but did not kill targets infected with *T. parva* (Marikébuni). This was based on results using target cells originating from four different KN104<sup>+</sup> animals. The targets consisted of bulk cultures and their derivative clones infected with either of the two parasite stocks, and a precloned T-cell population infected with either of the two parasite stocks (Table 2, Fig. 2).

**The Restricting MHC Molecule for the *Theileria*-Specific Cytotoxic Clones.** Immunoprecipitation studies on cells of B641 with alloantisera KNA104 and anti-w10, and mAbs IL-A4 and w6/32 confirmed that these antibodies precipitated class I MHC molecules—i.e., two polypeptides, 45 and 12 kDa (Fig. 3). Sequential immunoprecipitation of lysates of B641 cells with alloantiserum KNA104 and an anti-w10 alloantiserum (Fig. 3) showed that alloantiserum KNA104

Table 2. Parasite restriction and strain specificity of three cytotoxic T-cell clones from animal B641

| Cell line*  | Target                |                              | Class I MHC phenotype detected |                              |  | % cytotoxicity by clone† |         |         |
|-------------|-----------------------|------------------------------|--------------------------------|------------------------------|--|--------------------------|---------|---------|
|             | By allo-<br>antiserum | By mAb<br>IL-A4 or<br>KNA104 | By allo-<br>antiserum          | By mAb<br>IL-A4 or<br>KNA104 |  | T15.8                    | T15.114 | T15.132 |
|             |                       |                              |                                |                              |  |                          |         |         |
| B641 TpM    | w10/KN18              | +                            |                                |                              |  | 87                       | 81      | 84      |
| B641 TpM 1  |                       |                              |                                |                              |  | 73                       | 65      | 65      |
| B641 TpM 2  |                       |                              |                                |                              |  | 75                       | 68      | 67      |
| B641 TpA 17 |                       |                              |                                |                              |  | 7                        | 6       | 9       |
| B641 TpA 18 |                       |                              |                                |                              |  | 12                       | 8       | 8       |
| B641 blasts |                       |                              |                                |                              |  | 0                        | 0       | 0       |
| C234 TpM    | w10/w6                | +                            |                                |                              |  | 87                       | 87      | 89      |
| C234 TpA 12 |                       |                              |                                |                              |  | 10                       | 6       | 4       |
| C234 TpA 13 |                       |                              |                                |                              |  | 8                        | 8       | 7       |
| C234 blasts |                       |                              |                                |                              |  | 1                        | 0       | 1       |
| C167 TpM    | w10/w4                | +                            |                                |                              |  | 102                      | 96      | 99      |
| C167 TpA    |                       |                              |                                |                              |  | 2                        | 3       | 3       |
| C167 blasts |                       |                              |                                |                              |  | 0                        | 0       | 0       |
| T16.13 TpM  | w10/w7                | +                            |                                |                              |  | 106                      | 92      | 97      |
| T16.13 TpA  |                       |                              |                                |                              |  | 3                        | 3       | 4       |
| T16.13      |                       |                              |                                |                              |  | 0                        | 0       | 0       |

\*Cell lines are identified by the number of the donor animal or T-cell line, followed by the parasite strain identification if infected: TpM = Muguga, TpA = Marikébuni. *T. parva*-infected cell lines that have been cloned are identified by a number after the parasite strain. Blasts originated from cultures of Con A-stimulated blasts maintained in 20% TCGF. T16.13 is a cloned uninfected T-cell line from animal C447.

†Results are presented for an effector-to-target ratio of 1:1.

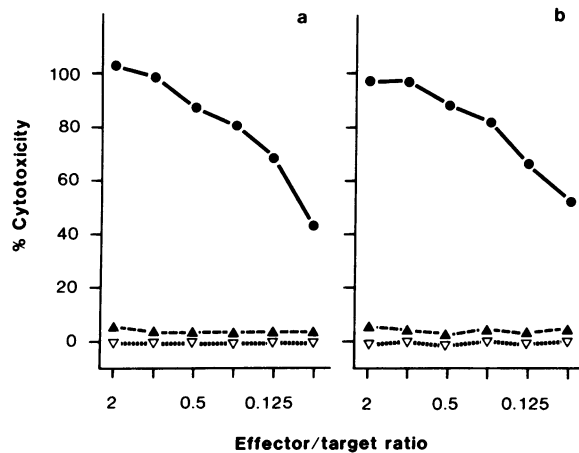


FIG. 2. Parasite restriction and strain specificity of cytotoxic T-cell clone T15.132. Effectors were harvested 9 days after last stimulation. Cytotoxicity was analyzed on infected and uninfected targets originating from bulk cell lines of animal C167 (a) or from precloned T-cell line T16.13 of animal C447 (b): ▽, uninfected cells; ●, cells infected with *T. parva* (Muguga); ▲, cells infected with *T. parva* (Marikébuni).

and anti-w10 alloantiserum recognized determinants on the same class I molecule from B641 cells. Similar results were obtained by using mAb IL-A4 followed by anti-W10 alloantiserum (not shown).

**Blocking of Cytotoxicity by MHC-Defining mAbs and Alloantisera.** To confirm the involvement of class I MHC molecules in restricting the cytotoxicity, experiments were conducted to test whether or not lysis could be blocked by preincubating the targets with antibodies recognizing the restricting class I MHC molecule. *T. parva* (Muguga)-infected cells from animal C603 carrying the w10 and KN104 specificities were used as target cells. Preincubation of C603 target cells with mAb IL-A4, alloantiserum KNA104, or mAb w6/32 reduced cytotoxicity by more than 90% (Fig. 1). Two other mAbs recognizing the target cells—namely, mAb BT3/8, which reacts with a non-MHC molecule (25), and mAb B1.1G6, which recognizes bovine  $\beta_2$ -microglobulin (24)—did not block the cytotoxicity (Fig. 1). Alloantiserum KNA109, which does not recognize the target cell, did not influence the killing (Fig. 1).

**Cell Surface Phenotype of the *Theileria*-Specific Cytotoxic Clones.** The three cytotoxic clones were BoT4<sup>+</sup>, BoT8<sup>+</sup>. When these effectors were incubated with mAbs IL-A12 or IL-A17 before inclusion in cytotoxicity assays, IL-A17 inhibited killing by 20–30%, whereas IL-A12 had no effect.

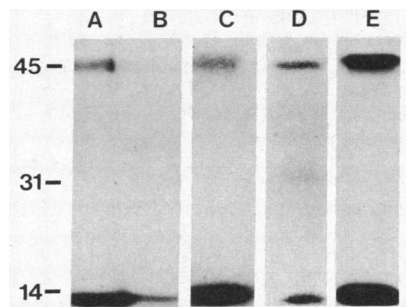


FIG. 3. Immunoprecipitation of Nonidet P-40 detergent extracts of cell surface <sup>125</sup>I-labeled Con A-stimulated blasts of animal B641. Sequential immunoprecipitation was performed with KNA104 (lane A), followed by an anti-w10 alloantiserum (lane B). Single immunoprecipitations were performed with anti-w10 alloantiserum (lane C), mAb IL-A4 (lane D), and mAb w6/32 (lane E).

## DISCUSSION

Three *Theileria*-specific cytotoxic clones were isolated from a population of PBM that had been stimulated five times *in vitro* with autologous *T. parva* (Muguga)-infected cells. These clones were parasite strain-specific and restricted by a class I MHC determinant.

The results of the present study extend previous observations on the immune responses of cattle to *T. parva* (6) and the related parasite *Theileria annulata* (27), which indicated that cytotoxic cells specific for parasitized lymphoblasts were probably restricted by class I MHC antigens. Cytotoxic activity of the three clones was restricted to targets bearing the KN104 specificity, which, in animal B641, is expressed on the class I MHC A locus-encoded molecule bearing the w10 specificity. This was confirmed by the ability of alloantiserum KNA104 and mAb IL-A4 to block lysis of the target cells. However, mAb w6/32, which reacts with a monomorphic determinant on the MHC molecule, was equally efficient at blocking cytotoxicity. Similar blocking of cytotoxicity by w6/32 has been reported with human cytotoxic cells specific for targets infected with influenza virus or Epstein-Barr virus (28–31). In these studies, it was suggested that blocking was due to steric hindrance by the mAb, to antibody-induced conformational alteration of the MHC molecule (28, 29), or possibly to interference in the association of the virus antigen with the MHC molecules (29). Similar mechanisms may account for blocking with w6/32 in the studies reported here.

The demonstration that the three *Theileria*-specific cytotoxic clones have the BoT8<sup>+</sup>, BoT4<sup>+</sup> phenotype further illustrates the similar functional properties of bovine BoT8<sup>+</sup> cells with human CD8<sup>+</sup> lymphocytes (31). The low level of blocking of cytotoxicity by mAb IL-A17 in the present study may relate to the relatively high avidity of the idiotype receptor of the cytotoxic T-cell clones for the target antigen, as has been suggested previously for blocking with mAb to Lyt-2,3 in mice (32), or to the epitope recognized by mAb IL-A17 being distant from the functional site on the BoT8 molecule.

Previous observations that bulk populations of *Theileria*-specific cytotoxic cells generated *in vivo* are biased in their killing towards targets infected with the parasite stock used for immunization (9) have been open to the criticism that target cell lines may vary in their inherent susceptibility to lysis, possibly as a result of infection of different cell types by the parasites. However, we have clearly demonstrated strain specificity of cloned effectors by using as targets a population of precloned T cells infected with two different stocks of the parasite. Cross-immunity trials have shown that *T. parva* (Marikébuni)-immune animals resist challenge with *T. parva* (Muguga), whereas fatal breakthrough infections occur in *T. parva* (Muguga)-immune animals when challenged with *T. parva* (Marikébuni) (5). The strain specificity of cytotoxic T-cell clones generated from an animal immunized with *T. parva* (Muguga), if representative of the response *in vivo*, may account for the failure to protect against challenge with *T. parva* (Marikébuni). Nevertheless, in bulk cultures of cytotoxic T cells derived from *T. parva* (Muguga)-immune animals by repeated stimulation of PBM *in vitro*, there is some killing of targets infected with *T. parva* (Marikébuni), suggesting that crossreactive populations of cytotoxic cells may exist (unpublished data). Variability between individual animals in the degree of protection conferred against challenge with *T. parva* (Marikébuni) by immunization with *T. parva* (Muguga) (5) may be due to the extent to which crossreactive effector cells are induced. By the same logic, one might expect cytotoxic cells generated against *T. parva* (Marikébuni) to be predominantly crossreactive. Generation of further cytotoxic T-cell clones against different parasite strains will be required to answer these questions. The

information obtained to date indicates some parallels with responses to influenza type A viruses in humans, for which virus subtype-specific and crossreactive cytotoxic T-cell clones have been demonstrated (33).

To our knowledge MHC-restricted cytotoxic T-cell responses to protozoan parasites have not been reported for parasites other than *Theileria* species (6, 27). Helper/inducer T-cell responses have been demonstrated in mice infected with *Plasmodium* and *Leishmania* parasites and have been shown to participate in the generation of protective responses (34, 35). In the case of *Leishmania tropica*, these T cells have also been cloned (35). However, generation of such responses appeared to require the presence of mononuclear phagocytic cells to process and present the parasite antigen, whereas *Theileria*-specific clones responded to parasite-induced changes on the membrane of the infected cell itself.

Cattle immunized against *T. parva* do not produce antibodies that react with the surface of parasitized cells (36). Furthermore, attempts to identify parasite products on the cell surface by using biochemical techniques and mAbs have so far been unsuccessful (6). Thus, at present, it is not possible to say whether the target antigen is of host or parasite origin, although the detection of parasite strain specificity on the part of T cells would favor the latter possibility. Similar problems in identifying the surface antigen recognized by the cytotoxic cells have been encountered with Epstein-Barr virus-infected and influenza virus-infected cells from humans (33, 37). In the latter, transfection of viral genes was required to provide definitive information on the virus molecules responsible for membrane changes recognized by the cytotoxic cell (33). Such an approach might be possible with *Theileria*-specific cytotoxic cells, should it become possible to identify the parasite gene(s) responsible for changes on the infected cell membrane, either directly or with mAbs recognizing the gene product.

In conclusion, the results of this study have demonstrated that cytotoxic T cells, which are restricted by class I MHC molecules and are parasite strain-specific, can be propagated and cloned *in vitro* from immune cattle. Findings on the specificity of these effectors, taken together with previous observations on cross-protection with different stocks of *T. parva*, suggest that these cytotoxic cells are important in mediating immunity to the parasite, and thus they may be of use, in conjunction with biochemical and molecular biological techniques, to identify antigens that could be used for immunization.

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